ToDo:

This is a Task Order contract with one option period starting 9/11/14. I need to inform (email) the CO (Ron Bell and/ or Nicole Hairston) of my intent to exercise the Option 1 for $89,933.00. This is the amount stated in the attached purchase order for the option period.

The base period for Phase I ends on 9/10/14. In EAS I need to do a PR to modify/amend contract to exercise option 1 starting 9/11/14 - 9/10/15 in the amount of $89.933.00. I will need to attach a statement of work for the Option Period. Since this is a Task Order contract, each task order must have a SOW. See Section 2 - Ordering by Designated Officers (Task Order guidance) and Section 3 - Option Periods of the attached Purchase Order for guidance.

This draft is currently under review by EPA personnel in OPP, ORD, and Region 5.

Phase 2 Statement of Work for Regional Applied Research Effort Perform Data Analysis and Create Experimental Design Concerning Bee Kill Incidents in the Upper Midwest Associated With Neonicotinoid Pesticide Treated Seed Plantings

I. Background:

EPA recognizes that honeybees (*Apis mellifera*) are important pollinators for the production of agricultural crops and resultant food production in the United States. Managed honeybee colonies have been in decline both in North America and Europe. EPA and the State Pesticide Regulatory agencies remain concerned about bee kill incidents (BKI) and any role that agricultural pesticides may play in these losses. A number of factors have been associated with declines including disease, pests, poor nutrition, loss of habitat, pesticides and bee management practices; however, none of these factors have been identified as the singular cause. Neonicotinoid insecticides are known to be highly toxic to insect and honey bees on an acute exposure basis. They are used on crops that require honey bees for pollination and/or on crops that may not require insect pollination but are nevertheless attractive to bees as a source of pollen and/or nectar. Efforts continue to be directed at determining the extent to which pesticides may be affecting bees and ways to mitigate potential effects. Due to their systemic activity, there is scientific evidence neonicotinoids move to some extent into pollen and nectar.

The Agency receives information on bee kill incidents through several channels, including required adverse effects reporting under FIFRA 6(a)(2), through the National Pesticide Information Center (NPIC), through the pollinator protection web page (beekill@epa.gov), from state reports and the general public. Based on information provided by beekeepers who have reported BKIs directly to EPA, there have been a number of episodes where BKI observations occurred at the same time that corn has been planted in the vicinity of the affected honeybee colonies. Some of the beekeepers who lost honeybees asserted that the corn seed planted adjacent to their colonies had been treated with neonicotinoid insecticides, specifically clothianidin (a common neonicotinoid). Research into mitigation of the effects of planter exhaust material from treated seeds on honey bees has established that many factors can potentially exert strong influence on the exposure of honey bees to dust. These factors include quality of

seed dressing, direction of exhaust, the active substance, timing of planting, climate conditions, crop type and treatment recipe (e.g., Forester et al. 2011, Marzaro et al. 2011).

The data sources for associating bee losses to agricultural practices occur at many scales, including national, regional, state, and farm levels and can be generated by regulators, beekeepers, farmers, and citizens. The available datasets consist of incident data from those investigating environmental or pesticide applications and their link to the bee kill incidents, including the states, EPA Regional and Headquarters offices, USDA, and Health Canada. Collation and analysis of the available data and appropriate statistical inference are necessary to establish what spatial association exists between the locations of bee kill incidents and nearby agricultural practices that can be inferred from land cover, weather data, pesticide use patterns, and crop coverage. Reference conclusions of Health Canada report and status of EPA data.

In addition, experimental evidence is needed under realistic field conditions that test the relationship between bee kill incidents and neonicotinoid exposure via pollen and corn dust from seed treatments. This includes information on exposures of worker bees in the field and the accumulation and persistence of neonicotinoids within the hive and to different life history stages within the hive.

II. Objective Statement: The objective of this contract is to collect relevant information concerning exposures and effects of neonicotinoids. Phase I focuses on utilizing available environmental data and pesticide use information collected during recent bee kill incidents in the Midwest and surrounding areas to assess predominant factors associated with these losses. A spatial-temporal analysis of weather, land type, and acreage planted and pesticide use will be overlain with the location of bee kill incidents and tested for spatial/temporal associations to the extent supported by available data. The results of this analysis could lead to improved forecasting and identification of exposure conditions conducive to bee kill incidents and improvements in agricultural planting practices that may avert similar incidents, and provide to OPP important information that may lead to pesticide label improvements and restrictions to further minimize adverse effects to insect pollinators. An experimental design will also be developed for field and controlled laboratory experiments in Phase 2 to test the hypothesized relationship(s) between measures of honeybee colony health (e.g., hive weight, population) and agricultural/environmental factors. The field/lab experiments will be used to support development of a colony simulation model that OPP is currently developing in collaboration with USDA; this model will serve as a means of estimating the potential effects of pesticides on honey bee colonies.

III. Phase 1 Task Description: This two-year project will be divided into two phases. Phase I will entail an analysis of incident data from the States, EPA Regional and Headquarter offices, US Department of Agriculture and Health Canada, looking for any environmental or pesticide application links to the BKIs. Phase 2 will utilize contractor or IGA support to collect environmental data (including missing data from year 1) that may be associated with the losses, and set-up a controlled field experiments in Region 5 States. In these field experiments all

parameters are measured before and after treated corn plantings nearby active, healthy, beehives.

The research goal for Phase I is to determine, based on available environmental and pesticide application data, whether neonicotinoid-treated seed plantings are responsible for BKI reported in the Upper Midwest. The hypothesis is that neonicotinoids in combination with as yet unknown factors such as environmental conditions or application method are responsible for the recently reported bee kill incidents. In Phase I, this hypothesis will be tested through geospatial and statistical analysis of incident investigation data collected/supplied to EPA by the State regulatory agencies, literature reviews, and publically available national and international data sources.

Phase 1 Task 1. Literature Review

From EPA Statement of Work:

Literature review task. Collate available data and conduct literature review to collect environmental and application data available about bee kill incidents to include North American beekeeper reported incidents. This database will support hypothesis testing through geospatial and statistical analysis of incident investigation data with project collaborators.

Ohio State Phase 1 Workplan Response:

A literature review and meta-analysis will be performed to determine what is currently known in the published literature, news reports and government documents related to bee kill incidents related to planting of seed treated with neonicotinoid pesticides. The scientific literature search will be conducted using ISI Web of Science and news report search will be conducted in Google web searches and using LexisNexis. Additionally, a literature review of current scientific reports on the effects of pesticide exposure to individual bees and whole bee colonies will be conducted with the goal of determining 1. the neonicotinoid exposure levels needed to impact colony health and 2. the particular aspects of colony health that could be measured to detect the effects of neonicotinoid seed treatment dust exposure on colony health and future productivity.

Phase 1 Task 2. Database Construction

From EPA Statement of Work:

Database construction. Make data collected under [Literature Review Task] available in an electronic database to collaborators.

Ohio State Workplan response:

Data from Bee Kill Reports will be input into a structured database managed in Microsoft Access in consultation with collaborators at USEPA and a statistician.

It is expected that much of the data needed to characterize the site at the time of the bee kill incident (weather information, accumulated degree days, status of corn planting in the region, surrounding land use) will need to be discovered or generated as a part of this project. Historical weather data will be collected from Accuweather and used to characterize the weather at the

time of the incident as well as the growing degree days accumulated for the year at the site. Growing degree days will be used to estimate the floral assemblage blooming at the time of the incident using the Ohio Growing Degree Day Phenology Calculator (http://oardc.osu.edu/gdd/).

Planting dates at each site will be estimated, where needed, using historical planting progress data published weekly by USDA-NASS for each state.

Land use around each Bee Kill Incident site will be categorized using ArcGIS and the USGS land cover database as well as crop coverage data for each year available from USDA-NASS. Different field crops, residential development, forest and roadways will be characterized at a 3 kilometer radius from the site of the kill incident.

Phase 1 Task 3. Design of the Phase II Field Experiment

From EPA Statement of Work:

Work with project collaborators to design a field experiment that tests whether neonicotinoid-treated seed plantings are responsible for BKI reported in the Upper Midwest.

Ohio State Workplan response:

Design of the field experiment will follow logically from the findings in Tasks I and II. The goal will be to design a robust field experiment that uses a minimum number of colonies in a minimum number of locations to provide the environmental and land use variability needed to test the association between the planting of treated seeds, local climatic conditions, plant phenology and the success of honey bee colonies.

Phase 1 Milestones & Deliverables (Contract Award Date was sometime in October 2013)

1. Milestone: Within 6 months of being awarded the contract (***April 2014***) and being provided access to the EPA BKI data, the contractor will collate available EPA data with other identified data sources and communicate this data set back to EPA. This literature review and data collection will include US, Canadian, State, and beekeeper reported incidents. The contractor will report results of this data synthesis effort by email to ERD, with supplemental metadata concerning database construction and descriptive statistics concerning the location of identified bee kill incidents.

2. Milestone: Within 9 months of being awarded the contract (***July 2014***) and being provided access to the EPA BKI data, the contractor will provide the results of the spatial analysis of the collated bee kill incident data. This analysis will be conducted in collaboration with relevant EPA personnel from Region 5, OPP, and ORD. The contractor will report results of this analysis by email to ERD and include any significant associations between the location of reported bee kill incidents and agricultural covariates.

3. Milestone: Within 9 months of being awarded the contract *(**July 2014***), the contractor will provide a detailed study design to implement a field experiment testing neonicotinoid exposure and bee colony health metrics. This study design will be completed with contributions of relevant EPA personnel from Region 5, OPP, and ORD. The contractor will report the study design by email to ERD.

4. Deliverable: Within 12 months of being awarded the contract (***October 2014***), the contractor will provide a short final report documenting the milestones that have been completed for Phase I of the contract. The contractor will provide an electronic copy of the final report by email to ERD.

Phase 1 Acceptance Criteria

1. Data compilation documentation should also include short reasoning on identified data products identified in the literature review that are not incorporated into the electronic data submittal.

2. The spatial analysis documentation should present significant and non-significant spatial associations in table form in the accompanying documentation.

3. The study design incorporates responses to comments from relevant EPA personnel from Region 5, OPP, and ORD.

4. While the final report is acceptable without an interpretation of the results with respect to recommendations for Best Management Practices and possible mitigation efforts to minimize honeybee colony losses, any such interpretation is encouraged.

IV. Phase 2 Task Description (Assumed award date of November 2014)

The research goal for Phase 2 assumes that the hypothesis that neonicotinoids are at least partially responsible for reported bee kill incidents has been confirmed. Phase 2 will continue Phase 1 efforts as appropriate to collect additional reported environmental data (including missing data from year 1) that may be associated with the losses. However, the primary focus of Phase 2 is to set-up controlled field/laboratory experiments in Region 5 States. In the field experiments, parameters of interest to the EPA are measured before and after treated corn plantings nearby active healthy, beehives and in a controlled experimental setting. An experimental design will also be developed for a field experiment in Phase 2 to test the hypothesized relationship(s) between measures of honeybee colony health (e.g., hive weight, population) and agricultural/environmental factors identified in the first year of the study. In the controlled lab experiment, doses will be given at predetermined levels to allow for measurements in different hive media and different bee life history stages. The field and lab data will be used to support development of a colony simulation model that OPP is currently developing in collaboration with USDA; this model will serve as a means of estimating the potential effects of pesticides on honey bee colonies. The phase 2 will be divided into 3 tasks, experimental design/quality assurance, field collection, controlled lab study.

The colony simulation model is based on a proposed framework for assessing risks of pesticides to bees (EPA/HC/CalDep White Paper 2012). The proposed framework is similar to risk assessments used by EFED for other taxa, relying on a tiered approach that starts with laboratory-based studies conducted at the individual level and increases in complexity to semi-field and field testing when risks are not precluded at the lower tiers. Tests conducted in laboratories are useful in assessing effects of pesticides on individual bees; however, they do not necessarily account for effects at the colony-level. Therefore, semi-field and field studies

involving exposures of entire honey bee (*Apis mellifera*) colonies to pesticides applied to crops are used to assess effects at the colony level. Even these study results of those studies can be difficult to interpret due to several variables, including pests and diseases of honey bees, nutritional status, weather and landscape, and bee management practices.

An additional model tier was therefore also included that simulates the processes of a honey bee colony and impacts on the colony following pesticide exposures of individual bees. This can facilitate consideration of the unique social structure of honey bees as well as the multiple stressors faced by honey bees. The Science Advisory Panel review suggested that model development could involve use of whole models or components of models (analytical models) that may be used to understand important colony processes. EFED selected the BEEPOP model (DeGrandi-Hoffman *et al*. 1989) with modifications to consider varroa mite infestation and treatment (DeGrandi-Hoffman and Curry 2004) as a baseline model that will be supplemented with pesticide exposure and effects data. Co-development of the pesticide module between the EPA and the USDA has revealed a number of areas where additional data on parameter estimation and algorithm selection were needed to fully implement the model. The field and controlled laboratory experiments as part of the Phase 2 scope are designed to inform these aspects of the model.

Phase 2 Task 1 Experimental Design/Quality Assurance (Jan-Feb 2015)

A Quality Assurance Project Plan (QAPP) will be created that contains the study design for the field and laboratory experiments. The QAPP should reference Standard Operating Procedures (SOPs) that list detailed steps that describe data collection techniques and methods of analysis for the collection of field data and the implementation of the closed nuc laboratory study. SOPs (EPA or Ohio State) should be referenced and provided as appropriate for hive maintenance, pollen identification, tissue residue collection and storage, and closed nuc dose implementation.

Phase 2 Task 2 Hive-level Field Exposure Experiment (Spring-Summer-Fall 2015)

Six spatial locations will be selected for hive-level field exposure experiments. Spatial locations are selected based on at least 40% coverage of agricultural cropland (planted in corn or soybeans) within 3km of the hives, based on the 2013 USGS Crop Layer database. Apiaries will consist of at least 6 overwintered colonies in clean hive boxes. Colonies are supplemented with sugar syrup, pollen and pollen substitute as needed following standard apicultural practices, but any protein supplementation ends on April 10 or two weeks before corn planting is expected to begin. If 6 overwintered colonies are not available at each site, new hives are established from package bees at least 2 weeks before corn planting season. Package colonies are provided with at least 2 frames of brood to speed build-up. After any new colonies are established, they will continue to be fed sugar syrup, as needed, until corn planting begins

Corn planting in Ohio typically occurs over a week period between April 15 and June 1, depending on weather conditions and soil moisture. Colonies will be monitored over a 2 month period of time associated with the planting and growing season, approximately from April 10

through June 15. Relevant tissue residue data and other hive-level variables will be collected. Data products include:

1) Pollen identification time series. Data will be collected to determine floral resources utilized by honey bees over time, establishing a pollen analysis time series with respect to proportion of pollen collected by bees for each of the 6 spatial sites. Bottom-mounted pollen traps will be used on 4 colonies at each site and pollen will be collected biweekly during corn planting and weekly during other periods. Collected pollen will be pooled by site. For each site pollen species will be identified using standard microscopic or colorimetric methods and summarized in an electronic data set.

2) Field collected pollen residues for neonicotinoid analysis. Pollen collected during corn planting will be separated at an appropriate taxonomic group/species level and prepared, stored, and shipped to EPA/ORD/Athens in a manner consistent with later extraction and analysis of neonicotinoid concentrations. Bulk unsorted pollen collected 1 week before and 2 weeks after corn planting will also be analyzed for neonicotinoid concentrations. Approximately 110 pollen samples will be submitted for neonicotinoid analysis.

3) Collect relevant data concerning the local area management and metrics to assess the health of honey bee colonies. This subtask includes collecting available local agricultural management information concerning local sources of pesticide applications, where possible, including formulations, timing, amounts from University land and any cooperating farms. Collect time series abundances of dead bees collected weekly and disease/load indicators. Weekly/monthly hive weights as practical.

4) Samples of bees, bee bread, honey or nectar, and larvae will be collected from 2 hives, not included in the pollen trapping study, at the same 6 sites. Samples will be pooled by site. Samples will be collected 4 times, once before corn planting, once during corn planting, and twice weekly after corn planting. Sample location, time, abundance (for bees) and weight will be recorded. Samples will be frozen after collection and sent to EPPA/ORD/Athens for extraction and analysis of body burdens. The total number of samples expected to be analyzed is approximately 102 samples from bees (24 samples), bee bread (24 samples), honey or nectar (24 samples), bee larvae (24 samples) and royal jelly, if queen cells are found during this period (6 samples).

Phase 2 Task 3 Closed Nuc Laboratory Study (Spring-Summer-Fall 2015)

Traditional honey bee bioassays use cohorts of similarly aged individual bees, either larvae or adults, to which known doses of toxicant are delivered. These individual-level experiments give researchers good experimental control, but often result in experimental conclusions which are difficult to extrapolate to the hive level. The inability to predict hive level outcomes from laboratory experiments is easily understood given the basic biology of honey bees. Honey bees are eusocial organisms that live in complex, caste differentiated societies with various pheromones affecting individual physiology through inter-caste and intra-caste mechanisms. However, experimentation at the hive level can be expensive, laborious, and difficult to obtain conclusive data with due to the nature of beekeeping, bees large foraging range and the inherently large variability between hives.

This task will use closed nucleus colonies as a bioassay. The bioassay will be conducted to expose nurse bees and larvae to defined levels of toxicants in a colony-like setting, but differs from a real colony in that exposure occurs within a closed box and no foraging is allowed. This method allows for sampling of pollen to determine the degradation of toxicants inside the hive in "bee bread" and can be used to assess the toxicokinetics of compounds as they move from contaminated pollen, through nurse bees and into larvae. The bioassay is based on and experiment outlined in Johnson and Percel (2013) which investigated the effects of fungicide exposure on developing queen larvae. Closed 4-frame nucleus boxes can be used to house cohorts of young worker bees along with queen pheromone strips to mimic a queen-right colony environment. Frames of recently laid eggs will be introduced along with frames containing purposefully contaminated, homogenized pollen for larval and nurse bee sustenance. Additionally, sucrose syrup will be fed as a carbohydrate source. The amount of pollen and sucrose syrup consumed during the experiment will be monitored, and brood rearing success (frame weight, uncapped brood versus capped brood as measure of brood survivorship) and general mortality will be assessed. Closed nuc bioassays will be conducted using control pollen, as well as pollen artificially contaminated with 4, 40 and 400 ppb clothianidin. Nucs with each treatment will be replicated at least 3 times, for at least 12 nuc box treatments in total.

This method will be used to test tissue residues and estimate toxic effects on nurse honey bees and larvae in a hive-like environment. This process consists of caging a queen in the nuc, preparing pollen frames with applicable pesticide dose, adding queen and nurse bees to egg frames, moving egg frames to an incubating colony, and then capturing emerging bees. Data, such as frame photos (larvae, capped brood, eggs and pollen), frame weights and emerging bee weights will be recorded electronically. Tissue samples for hive elements, including nurse bees, larvae, and bee bread, will be collected during setup (nurse bees and pollen), and at 4 days (larvae, nurse bees and pollen), 8 days (capped brood, nurse bees and pollen) and 12 days (capped brood, nurse bees and pollen) after setup. Approximately 132 samples will be collected in total. Samples will be frozen after collection and sent to EPPA/ORD/Athens for extraction and analysis of neonicotinoid body burdens.

Phase 2 Milestones & Deliverables

1) Milestone: QAPP and SOPs. Within 4 months of being contractor will provide a detailed study design with a QAPP and relevant identified SOPs. The contractor will report the study design by email to ERD.

2) Milestone: Electronic data delivery. Within 12 months of being awarded the contract (***October 2015***), the contractor will collate relevant data collected in an electronic format and communicate this data set back to EPA.

3) Milestone: Tissue residue samples. Within 12 months of being awarded the contract (***October 2015***), all relevant collected tissue residue sample will have been shipped to EPA/ORD/Athens.

4) Milestone: Final report. Within 12 months of being awarded the contract (***October 2015***), the contractor will provide a short final report documenting the milestones that have been completed for Phase 2 of the contract. The contractor will provide an electronic copy of the final report by email to ERD.

Phase 2 Acceptance Criteria

1)

2)

3)

4)

Supplemental Information: Neonicotinoid Analytical Support provided by EPA/ORD/Athens

ERD (Matthew Henderson's lab) will conduct both targeted and non-targeted pesticide analysis in various matrices (i.e. bee, honey, bee bread, and pollen) shipped to Athens in support of this Phase 2 Statement of Work. Samples received from the project's Principal Investigator (PI) will be inspected upon receipt for integrity and stored at a nominal temperature of -80°C. A sample log will be maintained with information about each sample received such as date of arrival, sample physical state (whether frozen or not), name and initials of person receiving samples, etc. *S*amples will be determined as suitable for analysis or not. Remaining samples after analysis will be stored frozen at -80°C until the completion of the project.

The method to be applied uses an extraction and cleanup technique based on solid-phase extraction, followed by analysis using liquid chromatography-tandem mass spectrometry. The method has been successfully validated at different concentration levels in bees, bee pollen and honey. Method validation data, including method description, instrumental parameters, estimated limit of detection (LOD) and limit of quantitation (LOQ), standard deviation of replicate recoveries, and other QA/QC data were previously reported and published in the open literature (Kamel 2011). Analytical reference standards of neonicotinoids and their metabolites shall be obtained from US EPA National Pesticide Standard Repository (NPSR), if possible. If standards are not available at the NPSR, other sources will be sought. Each standard will be accompanied by a valid certificate of analysis (COA), providing traceability and current expiration date.

Analysis of samples will start after verification of previous validation criteria by analyzing standards and matrix spike samples. Following pesticide extraction using modified versions of the QuEChERS procedure, selected neonicotinoids will be analyzed on an Accela HPLC system coupled to a Thermo TSQ Quantum Ultra Mass Spectrometer (Thermo Scientific). A LC-MS/MS method has been developed to allow chromatographic separation and subsequent quantification of seven neonicotinoids with instrument detection limits in the sub-ppb range. Non-targeted screening for pesticides will be conducted utilizing gas chromatography coupled with mass spectrometry on a Pegasus 4D GCxGC-Time of Flight Mass Spectrometer (LECO Corporation).

New advances in GCxGC technology allow for the separation of numerous co-eluting compounds that would occur in single dimensional analysis (i.e. GC/MS). Non-targeted approaches will allow for the detection of trace analytes potentially present in these environmental samples.

References

DeGrandiHoffman, G., S. A. Roth, G. L. Loper, and E. H. Erickson. 1989. Beepop - A Honeybee Population- Dynamics Simulation-Model. Ecological Modelling 45:133-150.

DeGrandi-Hoffman, G. & Curry, R. (2004) A mathematical model of Varroa mite (Varroa destructor Anderson and Trueman) and honeybee (Apis mellifera L.) population dynamics. International Journal of Acarology, 30, 259–274.

Johnson, Reed M., and Eric G. Percel. “Effect of a Fungicide and Spray Adjuvant on Queen-Rearing Success in Honey Bees (Hymenoptera: Apidae).” *Journal of Economic Entomology* 106, no. 5 (October 1, 2013): 1952–57. doi:10.1603/EC13199.

Kamel, A. 2011. Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromotography-tandem mass spectrometry (LC-MS/MS). Journal of Agricultural and Food Chemistry.

Kamel, A, 2012. Quality Assurance Project Plan: Determination of Residues of Imidacloprid and its Metabolites in Bee, Bee Bread, Bee Larvae, Honey, and Royal Jelly Samples. ACB Project B12-03. EPA/OPP/BEAD/ACB.

White Paper in Support of the Proposed Risk Assessment Process for Bees. Submitted to the FIFRA Scientific Advisory Panel for Review and Comment. September 11 – 14, 2012

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End of revised statement of work for Phase 2

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Supplemental information below.

Text from Sole Source Justification with Dr. Reed Johnson Ohio State University Lab

1. Sole source justification under the authority of FAR 6.303-1 (a) (b) – Only one responsible source and no other supplies or services will satisfy the agency's requirements.

2. The proposed contract calls for a significant range of scientific capabilities on a relatively limited budget, including microscopic and DNA skills to identify pollen sources, active management of bee hives to enable exposure studies, chemical analytical capabilities to detect trace amounts of pesticides in pollen and in hives, access to corn fields to enable dust-off experiments, and even a public outreach component. In addition, the project requires that the research must be performed in an EPA Region 5 state. Based on current market research, The Pollinator Toxicology Lab in the Entomology Department at The Ohio State University, headed by Dr. Reed Johnson, is located at the Ohio Agriculture Research and Development Center in Wooster, OH and is the only research lab in the Region 5 states that possesses all of the above capabilities required to successfully investigate the effects of corn seed-treatment dust on honey bee hives in the field. The ongoing research of the lab focuses on protecting pollinators from pesticides and toxins. They have extensive expertise in investigating the effects of pesticides on honey bees and native pollinators across multiple levels, including the genomic and sub-lethal behavioral levels, as well as the effects of multiple stressors. Based on our market research, the other bee researchers in Region 5 focus on protecting pollinators from diseases specifically or have only examined the effects of pesticides on bees from the exposure angle. Members of the Ohio Agricultural Research and Development Center lab have the requisite practical beekeeping expertise to move, manage and assess the health of honey bee colonies placed within range of fields being planted with seed-treated corn. The lab currently manages 20 honey bee hives that are immediately available for use in field experiments. Members of the lab also have years of experience assessing pesticide concentrations in bees using analytical chemistry tools, including GC-MS, Reversed Phase HPLC and LC-MS-MS, and have access to equipment for performing these analyses within the department that are required to satisfy the statement of work. Lab members also have experience with pollen analysis, which will be a key skill in determining the floral resources utilized by honey bees during the corn planting season which is also necessary under the statement of work. The lab is currently conducting a survey of beekeepers in Ohio for which lab members were required to complete Institutional Review Board training on the handling and collection of location data and have experience performing spatial analyses using GIS, all skills called for in the statement of work. Dr. Johnson is currently planning a preliminary experiment for April 2013 in which pollen will be trapped, identified and analyzed from colonies foraging among the corn-dominated areas in central Ohio. Therefore,

this lab is uniquely capable of performing all the elements of the scope of work presented in the contract. Based on our market research, this lab is the only lab in Region 5 that has the expertise, experience, knowledge and unique capabilities to perform the work and meet the specifications stated in the statement of work.

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Integrated responses to questions for EPA participants, EPA Participants contribute below

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Phase 1 Task 1 Questions:

1) What references and databases are we aware of that document North American bee kill incidents?

The EPA has collected ~279 articles from the peer-reviewed literature related to honey bees, honey bee exposure to pesticides, and neonicotinoid pesticides. This literature is stored in a dropbox account folder and has been organized in an Endnote database.

National level databases include the United States Bee Kill Incident reports (spring 2012, n=24) collected from the National Pesticide Information Center (http://pi.ace.orst.edu/erep/) as well as the bee kill incident reports from Canada (spring 2012, 40 beekeepers, 240 bee yards) from Health Canada Pest Management Regulatory Agency (PMRA). State level databases that document North American bee kill incidents have not been fully investigated however there are some possibilities such as:

Washington state has an incident reporting place but does not have 2012 data on their website (http://www.doh.wa.gov/DataandStatisticalReports/EnvironmentalHealth/Pesticides.aspx).

Phase 1 Task 2 Questions:

1) What covariate databases are we aware of that may be helpful for spatial/temporal analyses of bee kill incidents?

Weather data (precipitation, temperature, wind speed, dew point, relative humidity) can be downloaded from NOAA - National Climatic Data Center. Land cover data is available for the whole U.S. from the NLCD 2006 layer (http://www.mrlc.gov/nlcd06_data.php). USDA National Agricultural Statistics Service publishes crop maps and statistics. The information from 2012 is available for download (http://www.nass.usda.gov/research/Cropland/Release/). Road and street information can be obtained from the Tiger files created by the US Census Bureau (http://www.census.gov/geo/maps-data/data/tiger.html).

Phase 1 Task 3 Questions:

1) What is our understanding of Johnson lab/field capabilities?

The Johnson lab has 20 bee hives that can be utilized immediately in a field experiment. They also have the capability to identify pollen species from bees or a hive using microscopic and genetic methods. The Johnson lab has years of experience doing research on honey bee exposure to pesticides and training and experience handling surveys and human subjects.

2013 work: colonies in association with planted crops, dead bee traps with bees collected in 2013 spatial analysis defined with colony home range (0.5 mile-3km radius?) and landscape/land use characterization (land use/abundant/scarce /no bloom/forest/residential/road), drift to dandelions on agricultural field margins; mustard/mint exposures within the fields, safe zone versus risk zone concept (proportions) for neonic dust deposition within a colony range

time series of dead bees associated with deposition rates; weekly data

usda-ams gastonia lab for residue analysis- significant amount of seed treatment neonics

pollen analysis times series with respect to proportion of pollen collected by bees week-by-week for 3 sites

active ingredient concentrations by pollen type via the Gastonia lab

control of weeds in the field during the time of bee planting a big deal as a source of neonic residues in the field

effects of insecticide exposures:

-15 of 18 colonies survived to June 15 and appeared to be doing well (colonies that did not survive probably due to queen issues, not pesticides)
-Beekeepers did not report bee kills (exposure was probably very similar – 20-50 ppb clothianidin)
-38 ppb clothianidin (oral) affected foraging efficiency (Schneider et al., 2012)
-“Sub-lethal” effect of exposure?? -- might be the target of phase 2 type analyses

2014-2015 possibilities:
repeat field experiments of bloom sources, pollen residue levels and dead bee traps with lubricant instead of talc as source of dust-off (already being done, unsure whether local farmers are switching to lubricants)
uncapped brood versus capped brood as measure of brood survivorship

photographic analysis of brood survival

transition to foragers, paint-marked cohorts to get at age to first foraging- measure of sublethality- could be an internal prediction of bee models

2) What are sources of neonicotinoid bee exposure in the field and how can we measure sources of exposure in the field?

Bees can be exposed through dermal contact with and consumption of pesticide via multiple pathways in the field. Crops can be treated with neonicotinoids by spraying or seed treatment. Contact exposure can occur from a bee flying through pesticide in the air, pesticide that has landed on a plant, or on the soil. Contact exposure during flight can occur during planting of seed treated corn or during spray application. Oral intake of pesticide can occur if a crop has been recently sprayed and a bee consumes the nectar or pollen. In the case of neonicotinoids in seed treatments, exposure can occur by consumption of nectar, pollen, guttation fluid of the

treated plant during its lifespan (Reetz et al. 2011). Contact and oral exposure is not limited to the areal extent of the treated crops field. Dust from seed planting or drift from spray application can drift onto the soil, plants, and surface water adjacent to the treated field (Krupke et al. 2012). Exposure to neonicotinoids via the soil from previous applications as much as a year previous are possible (Krupke et al. 2012).

Seed treatment coating rates are 0.25-1.25 mg clothianidin or thiamthoxam per seed. Air-powered seeders generate air concentrations and pollen depositions leading to exposure. Talc/graphite currently being swapped out with lubricant to reduce aerial exposures.

3) How can we confirm that bee exposures to neonicotinoids have occurred in the field?

The field study could be planned as a semi-field or tunnel experiment. A semi-field experiment places both the bees and the crops in an enclosure structure like a greenhouse so the area of where the bees are foraging is limited to the crop of interest. Other experiments have put the bees in cages along planting routes. Another method of ensuring exposure it to train bees to fly over a certain field to get to a feeding station. Biomarkers could be used to assess the stress response of an individual bee as well as determine exposure to a certain class of pesticides (Badiou-Bénéteau et al., 2012 and Carvalho et al. 2013).

4) How can we experimentally measure effects at the individual organism, population, and hive (structure) levels from neonicotinoid bee exposures?

Forager mortality and forager activity could be monitored by individually tracking bees with RFID tags and automated sensors. Brood and worker numbers can be measured empirically by measuring weight or area covered or subjectively through visual inspections. Alternatively, newer computer assisted digital image analysis (e.g. using Image J software to measure number of bees and area of brood) could be a good middle ground because it is less invasive than other empirical measures of weight or area but is still relatively accurate.

5) What predictions of exposure and effects endpoints can be made by the modified USDA VarroaPop model?

Exposure endpoints that can be made by the modified USDA VarroaPop model are split into worker and brood levels. Exposure endpoints will be measured as dietary doses for the different castes (worker, drone, queen) in a hive as developing larvae. Dietary doses for adult bees performing different tasks including 1. cell cleaning and capping, 2. brood and queen tending, nurse bees, 3. comb building, cleaning and food handling, 4. foraging for pollen, 5. foraging for nectar, 6. maintenance of hive in winter, 7. drone, and 8. queen. As far as contact exposure, an exposure endpoint of the model is forager bees from foliar application.

The proposed pesticide module white paper did not go into effects in much detail, mainly how the exposure was modeled was discussed but now how the exposure will be modeled as an effect. However, there are a number of different ways that both acute and chronic exposure to pesticides can be modeled in the modified VarroaPop model as having effects on bees among different castes. Endpoints for individuals from field and semi-field experiments could be number of foragers, foraging bout frequency, amount of pollen collected, duration of pollen foraging

bouts, forager mortality. Endpoints for the colony from field and semi-field experiments could be number of workers, number of brood, queen cell production, nest structure mass, worker mortality, worker loss, disease burden, and colony failure. Individual effects endpoints that could be modeled with the modified VarroaPop model include number of foragers and forager mortality. Colony level effect endpoints that could be predicted with the model include number of workers, number of brood, worker mortality, worker loss, disease burden, and colony failure.

6) How can we compare predictions from the model to what was measured in the field experiments?

MCMC

7) Design implications…Sample size

Phase 2 Task 1 Questions

Phase 2 Task 2 Questions

Phase 2 Task 3 Questions

Closed Nuc Controlled Exposure Design

Red carpet protocol:

Queen caging

1. Find 6 empty drawn out black Pierco or wooden frames.
2. Place the frame on a flat surface with the top bar facing away from you.
3. Lay the wooden T-shaped pin-guide on top of the frame so that the top of the T is against the inside of the left-hand side of the frame.
4. Place a red map pin in the hole on the left and a green map pin in the hole on the right.
5. Lift the T-shape off of the frame and place a yellow pin in the cell diagonal from the red pin on the lower right-hand side. Repeat with a blue pin diagonal from the green pin.
6. Prepare top and bottom barcodes for each hive. Each frame should have two barcodes; one for the top of the frame and one for the bottom of the frame. The arrows on both the top and bottom barcodes should be pointing towards the pins. The “bottom” barcodes should be placed on the top bar with the arrows pointing toward the comb and the ‘top’ barcodes should be placed on the bottom bar of the frame with the arrows facing up. Attach the barcode using hot glue. Staples can be used on wooden frames but make sure not to staple areas with barcodes or writing.
7. Once a queen is found in a colony carefully place her on the pinned frame and mark her on the thorax with a paint pen, if needed. Put the cage over the queen and press down

so that there are no holes between the wax and the cage. Make sure to leave enough space for the queen to move easily under the cage. Return frame to the host colony near frames of brood and leave enough space between the cage and the frame next to it for worker bees to get through.

Preparing pollen frames

8. Locate six empty drawn frames
9. Measure 90 grams of pollen and pour it into the Ninja food processor.
10. Add 5ml of distilled water and 5ml of diluted pesticide to the food processor and blend for thirty seconds. After 15 second scrape the sides of the food processor into order to blend pollen more thoroughly.
11. Take a 1-2 gram sample of pollen from each treatment used and put it in the freezer.
12. Weigh the pollen frame before and after the addition of pollen.
13. Push pollen into one side of the frame by pouring the contents of the pollen onto the frame and rolling it into the empty cells with your hands. Use gloves to do this and make sure to switch gloves between treatment groups.
14. Spray the frame a few times with 1:1 sugar solution to help the pollen stay in the frame.
15. Photograph pollen frames using only camera 2 on the Red Carpet. Label the frame using pieces of paper. Zoom the camera to the appropriate field of view and take a photo by pressing the shutter-release button on the camera.
16. Nail a queen pheromone strip (BeeBoost, Pherotech) attached to a zip tie to the top of the pollen frame.

Collecting egg frames and nurse bees

17. Return to host colonies with caged queens 24 hours after caging and locate the queen.
18. Remove cage and release queen into the bottom hive box.
19. Shake two frames of nurse bees from upper boxes into bulk nurse bee box with a frame containing multiple pieces of queen pheromone frame. If hive is not strong take nurse bees from another hive but make sure not to shake the queen into the nurse bee box.
20. Use a spray bottle with 1:1 sugar solution to keep nurse bees from flying out of the box. Leave box in a shady area until the nurse bees are ready to be added to the hives.
21. Weigh egg frames and photograph them using the Red Carpet.
22. Place each frame into a closed plastic nucleus colony (BeeBrief) with respective pollen frame and empty division board feeder with #8 mesh screen inside..
23. Face eggs towards pollen.
24. Label nucleus hive box and lid with hive number, treatment, and date.
25. Weigh each empty nucleus hive box.
26. Add two scoops of nurse bees (approximately 1 pound or 350g of bees) into each hive box using yellow milk jug scoop.

27. Weigh hive boxes after the addition of nurse bees.
28. Take hives to the storage building and place a mason jar feeder on top of the screen lid of each hive. Make sure the holes of the feeder are directly over the division feeder in the hive so that leaking sugar solution will fall into the feeder below.
29. Mason jar feeders (1 qt.) should contain 250ml of 1:1 sugar solution and be replaced every 3-4 days.

Moving egg frames to incubating colony

30. Check control nuc to see if at least 75% of brood are capped.
31. If brood on the control frame are capped transfer all nuc boxes to a location where remaining bees can be released.
32. Take egg and pollen frames and leave nuc boxes open to allow remaining bees to fly out.
33. Photograph and weigh egg and pollen frames using The Red Carpet.
34. Take a 1-2g sample of pollen from each pollen frame and freeze it.
35. Put egg frames into incubating colony facing other brood frames.
36. Record which incubating colony each egg frame is located in.
37. Measure dead bees remaining in each nuc boxes.

Emergence

38. Take frames of brood out incubating colonies the day before emergence.
39. Weigh and photograph frames using the Red Carpet
40. Place each frame into a Rubbermaid cooler and place into incubator at 34˚C and 80% RH.
41. Weigh emerging bees and frame each day.
42. Take photo of frames using the Red Carpet.